U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-252 076

EFFECTS OF OZONE ON NITROGEN FIXATION IN LADINO CLOVER

North Carolina State University

PREPARED FOR
Environmental Research Laboratory

March 1976

124151

EPA-600/3-76-031
March 1976

PB 252 076

Ecological Research Series

EFFECTS OF OZONE ON NITROGEN FIXATION IN LADINO CLOVER

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Corvallis, Oregon 97330

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-76-031		PB252076

4. TITLE AND SUBTITLE	5. REPORT DATE
EFFECTS OF OZONE ON NITROGEN FIXATION IN LADINO CLOVER	March 1976
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Udo Blum and Michael Letchworth	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Botany Dept. North Carolina State University Raleigh, North Carolina 27607	1AA006 ROAP 21ALR Task 24
	11. CONTRACT/GRANT NO.
	EPA-IAG-D5-0416
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Corvallis Environmental Research Laboratory Ecological Effects Research Division Office of Research and Development U.S. Environmental Protection Agency Corvallis, Oregon 97330	Interim Final
	14. SPONSORING AGENCY CODE
	EPA-ORD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Research was performed to determine the effects of ozone on ladino clover growth, nodulation, nitrogen fixation, nitrogen content, phenol content, energy value and root exudates. Plants were exposed once or twice to carbon-filtered air, 30 or 60 pphm ozone for 2 hrs. The effects of ozone on growth, nitrogen fixation, % nitrogen, total nitrogen and nodulation varied with age of plant and ozone concentration. Caloric content of tops (plants 4 weeks old when exposed) was reduced with two exposures of 30 or 60 pphm ozone. Root caloric content was not affected. Phenol content of tops and roots (plants 6 weeks old when exposed) was reduced with two 30 pphm ozone exposures. Clover exudates were not detectably modified by ozone exposure.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
ozone nitrogen fixation plant growth air pollutants microorganisms soils	air pollution ozone exposure	51

PRICES SUBJECT TO CHANGE

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
LIMITED	UNCLASSIFIED	32
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	$4.00

EPA Form 2220-1 (9-73)

GPO 995-565

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-76-031
March 1976

EFFECTS OF OZONE ON NITROGEN FIXATION IN LADINO CLOVER

by

Udo Blum and Michael Letchworth
North Carolina State University
Raleigh, North Carolina 27607

EPA-IAG-D5-0416

Project Officer

Dr. L. C. Raniere
Ecological Effects Research Division
Corvallis Environmental Research Laboratory
Corvallis, Oregon 97330

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY
CORVALLIS, OREGON 97330

DISCLAIMER

This report has been reviewed by the Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

Research was performed to determine the effects of ozone on ladino clover growth, nodulation, nitrogen fixation, nitrogen content, phenol content, energy value and root exudates. Plants were exposed once or twice to carbon-filtered air, 30 or 60 pphm ozone for 2 hrs. The effects of ozone on growth, nitrogen fixation, % nitrogen, total nitrogen and nodulation varied with age of plant and ozone concentration. Caloric content of tops (plants 4 weeks old when exposed) was reduced with two exposures of 30 or 60 pphm ozone. Root caloric content was not affected. Phenol content of tops and roots (plants 6 weeks old when exposed) was reduced with two 30 pphm ozone exposures. Clover exudates were not detectably modified by ozone exposure.

This report was submitted for limited distribution in partial fulfillment of an Interagency Agreement by the Agricultural Research Service (ARS) under the sponsorship of the Environmental Protection Agency (EPA). The research included in the report was cooperatively sponsored by ARS, EPA and the North Carolina Agricultural Experiment Station. Work was completed as of June 30, 1975.

ACKNOWLEDGMENTS

Parts of this research were conducted in the North Carolina State Unit of the Southeastern Plant Environment Laboratories. The help of the staff is appreciated.

The assistance of Mr. Hans Hamann in statistical design and anaiysis is gratefully acknowledged.

CONTENTS

		Page
Abstract		iii
Acknowledgements		iv
List of Figures		vi
List of Tables		vii
I	Introduction	1
II	Conclusions	2
III	Recommendations	3
IV	Experimental Work	4
V	References	21

LIST OF FIGURES

Number		Page
1	The effect of two 2-hr. ozone exposures on the top dry weight of ladino clover plants.	7
2	The effect of two 2-hr. ozone exposures on the root dry weight of ladino clover.	8
3	Changes in nodulation of ladino clover plants following two 2-hr. ozone exposures.	9
4	Effects of ozone exposures on nitrogenase activity/nodule of ladino clover.	10
5	Changes in nitrogenase activity/plant of ladino clover following ozone exposure.	11
6	Effect of two 2-hr. ozone exposures on % Nitrogen of ladino clover tops.	12
7	Effect of two 2-hr. ozone exposures on % Nitrogen of ladino clover roots.	13
8	The effect of two 2-hr. ozone exposures on the total N content of ladino clover tops.	14
9	The effect of two 2-hr. ozone exposures on the total N content of ladino clover roots.	15
10	The effect of two 2-hr. ozone exposures on the total plant nitrogen of ladino clover.	16

LIST OF TABLES

Number		Page
1	Caloric Content, Top Dry Weight, Root Dry Weight and Nodule Number of Ladino Clover Exposed to Ozone or Carbon Filtered Air.	17
2a	Phenol Content, Nitrogen Content, Top Dry Weight, Root Dry Weight and Nodule Number of Ladino Clover Exposed to Ozone and Carbon-Filtered Air.	18
2b	Top Dry Weight, Root Dry Weight, Nodule Number, Nitrogen Content and Phenol Content of Ladino Clover treated with Root Exudates.	18
3	Top Dry Weight, Root Dry Weight, and Nodule Number of Ladino Clover exposed to ozone or carbon-filtered Air Grown Separately or in Combination (Design is such that plants interact only by way of root exudates).	19
4	Root Exudates vs *Rhizobium*	20

SECTION I

INTRODUCTION

The research presented in this report is part of a continuing cooperative project between the Agricultural Research Service, the Environmental Protection Agency and the North Carolina Agricultural Experiment Station. The title of the overall project is, "Effects, fates and transformations of selected air pollutants in plants, microorganisms and soils."

The primary objectives of this cooperative program are to understand the impact of air pollutants on plants, microorganisms and soils that are of importance to agriculture, and to assist other agencies in relation to their mission of protecting the agricultural segment of the environment. The research thrust is directed at comparative studies on vegetation effects under phytotron, greenhouse and field conditions. Emphasis is on: (1) dose-response curves; (2) the interaction of various factors on the response of the whole plant to air pollutants; (3) assessing the impact of controlled pollutant additions and ambient pollution on plant biomass, yield and quality in the greenhouse and field, and on pollutant uptake and transformations in the greenhouse; (4) acute and chronic screens; and, (5) varietal responses.

Research reported here contains, as its major thrust, part of the phytotron (controlled environment) portion of the foregoing cooperative program. It was determined that ladino clover should be intensively studied under controlled conditions. The results should then be verified under greenhouse and field conditions and using selected other plant species. Ladino clover was selected as one of the most important forage species of the Southeast. Its use is increasing, especially in conjunction with certain grass species. Ladino has chronic problems under field cultivation that are not understood - air pollution may be a factor. Ladino is a legume and thus one of the important nitrogen fixing crop species. These factors make it an ideal test species.

The specific objectives for this research are given in Section IV.

SECTION II

CONCLUSIONS

Effects of ozone on Ladino clover growth and nodulation varies with age of plant and concentration of ozone.

Nitrogen content of plants exposed to 60 pphm of ozone was reduced for all exposure ages. Nitrogen content of plants exposed to 30 pphm of ozone varied with exposure age.

Caloric content of tops (plants 4 weeks old when exposed) was reduced by two ozone exposures of 30 or 60 pphm. Caloric content of roots was not affected.

Phenol content and nitrogen content of tops and roots (plants 6 weeks old when exposed) were reduced with two 30 pphm ozone exposures.

Clover root exudates were not detectably modified by ozone exposure of clover.

SECTION III

RECOMMENDATION(S)

Present indications are that the reductions of ladino clover (a forage crop) growth, nodulation, nitrogen content, energy content, and phenol content, observed as a result of ozone exposures, are closely related to plant energy fixation and plant energy allocation. The effects of ozone on energy fixation and distribution need to be explored further. The following is recommended:

1. determination of ozone effects on carbohydrate content.
2. determination of ozone effects on metabolic pathways - ie glycolysis, carbon cycle, and monophosphate shunt.
3. determination of ozone effects on translocation.

SECTION IV

EFFECTS OF OZONE ON NITROGEN FIXATION IN LADINO CLOVER

Most soils continually lose nitrogen and thus require added nitrogen to maintain soil fertility. Leguminous crops are an important source of such nitrogen. The amount added by legumes depends on plant growth and vigor as well as certain edaphic factors (ie. nutrient levels, water availability, pH and temperature). Exposure of soybean, pinto bean, and ladino clover to ozone reduces growth and nodulation (1-3). How these relate to quantitative and qualitative crop production is uncertain.

The present research was undertaken with Ladino clover to determine the effects of ozone on: growth, nodulation, nitrogen fixation, nitrogen content, phenol content, energy value; and root exudates.

MATERIAL AND METHODS

Experiment 1

Inoculated seeds of Trifolium repens L. c.v. Tillman were planted in gravel and Jiffy Mix (2:1). Complete nutrient solution was added to pots every third day. Distilled water was given at other times. Plants were grown and exposed to ozone in the Southeastern Plant Environmental Laboratories. Growth chamber environment was as follows: 12 hrs at 45000 lux, the night was interrupted for 3 hrs with 5500 lux, temperature 22C day and 19C night, and a relative humidity of 50% during the day and 60% at night. Plants were subjected to two 2 hr exposures (one week apart) of either carbon-filtered air, 30 or 60 pphm ozone. Groups of plants were exposed at two week intervals to determine age effects. The first of 4 harvests for each exposure age occurred 1 week after the second exposure. Dry weight of tops (TDW), roots (RDW), nitrogenase activity and nitrogen content were determined.

Experiment 2

Plants were grown in sand and Jiffy Mix (2:1) for one month in a greenhouse. A 1/10 normal nitrogen nutrient solution was added every third day. Plants were exposed to carbon-filtered air, 30 or 60 pphm ozone for 2 hrs. Half of the plants were harvested one week later. The remaining plants were exposed again and harvested the following week. TDW, RDW, nodule number, and caloric content of tops (TPCAL) and roots (RTCAL) were determined in each case.

Experiment 3

Plants were grown in sand for 6 weeks and then exposed to carbon-filtered air or 30 pphm of ozone (2 hr) twice 3 days apart in a greenhouse. A 1/10 normal nitrogen nutrient solution was added every third day. Deionized water was added at other times. Root exudates were collected 7 days after the last exposure. One hundred ml was collected from each pot. Exudates were collected the day before nutrient application. This was done to minimize inorganic ion input into the exudate. The exudate was used to water 3 week old seedlings of clover for 14 consecutive days. All plants were then harvested. TDW, RDW, nodule number, nitrogen content, and phenol content (4) were determined.

Experiment 4

Plants were grown in sand culture with 1/10 normal nitrogen nutrient solution as described by Kochhar (1974). Four week old plants were exposed twice 3 days apart to carbon-filtered air, 30 or 60 pphm ozone. Plants were harvested 4 weeks later. TDW, RDW and nodule number were determined. Root exudates (100 ml) from each reservoir were flash evaporated and taken up in acetone (5 ml). Acetone fraction was tested for toxicity against Rhizobium with sensitivity disks.

All data was subjected to analysis of variance unless otherwise stated. Least significant differences at the 5% probability level were used to illustrate differences among treatment means.

RESULTS

Experiment 1

Growth and Nodulation--

Fig 1-3. A double exposure of ozone reduced top growth, root growth, and nodulation. Plant responses to ozone varied with age and concentration of ozone.

Nitrogenase activity--

Fig 4-5. Some stimulation of nitrogen activity per nodule was observed for exposure age 28+35 and 42+49. However, these responses were not significantly different from the controls. Nitrogenase activity per plant was reduced for exposure age 14+21.

Nitrogen content--

Fig 6-10 [Also see Table 2a] Percent nitrogen for tops and roots was significantly higher for exposure age 14+21 for both the 30 and 60 pphm ozone exposures. Percent nitrogen was higher for tops with the 30 pphm exposures and higher for the roots with the 60 pphm exposures for exposure age 28+35. Total plant nitrogen was reduced for exposure age 14+21 and 28+35 for both ozone exposures. For exposure age 42+49 and 56+63 only the 60 pphm exposures reduced total nitrogen content of clover plants. Similar trends were observed for nitrogen content of tops and roots.

Experiment 2

Energy Content--

Table 1. Caloric content was significantly reduced for the tops with 2 ozone exposures of 30 or 60 pphm. Caloric content of roots was not significantly reduced.

Experiment 3

Phenol content--

Table 2a+b. Exposures of ladino clover to 30 pphm of ozone significantly reduced phenol content of tops and roots. Nitrogen content was also determined here.

Nitrogen of tops and roots was significantly reduced from controls.

Experiments 3 and 4

Root Exudates--

Tables 2b, 3, 4. Exudates from ozone exposed plants did not inhibit the growth or nodulation of ladino clover seedlings when compared to control plant exudates. Exudates from ozone exposed plants were not inhibitory to Rhizobium when compared to controls.

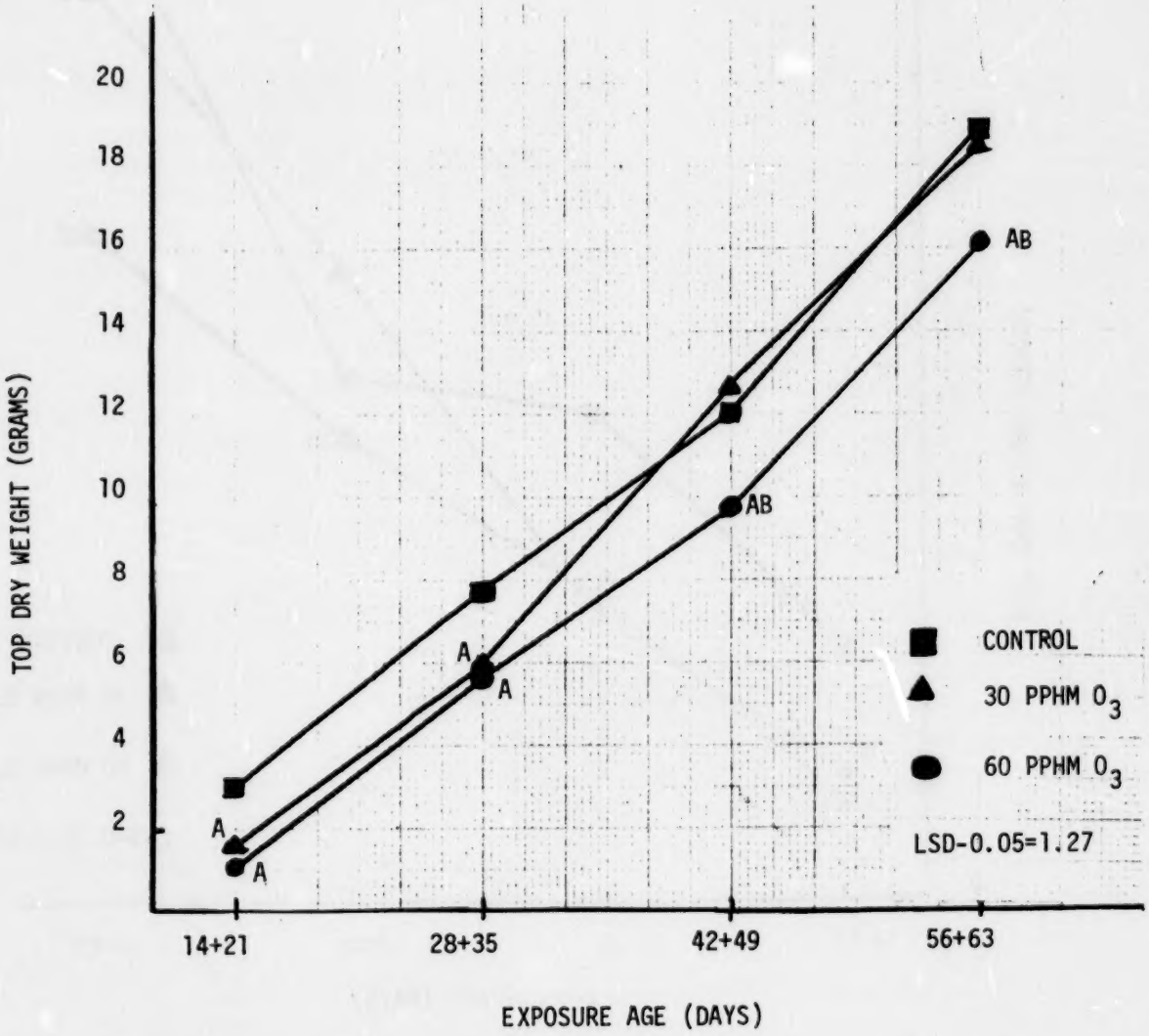

Figure 1. The effect of two 2-hr. ozone exposures on the top dry weight of ladino clover plants.

A.-significant difference between control and test.

B.-significant difference between 30 and 60 pphm ozone.

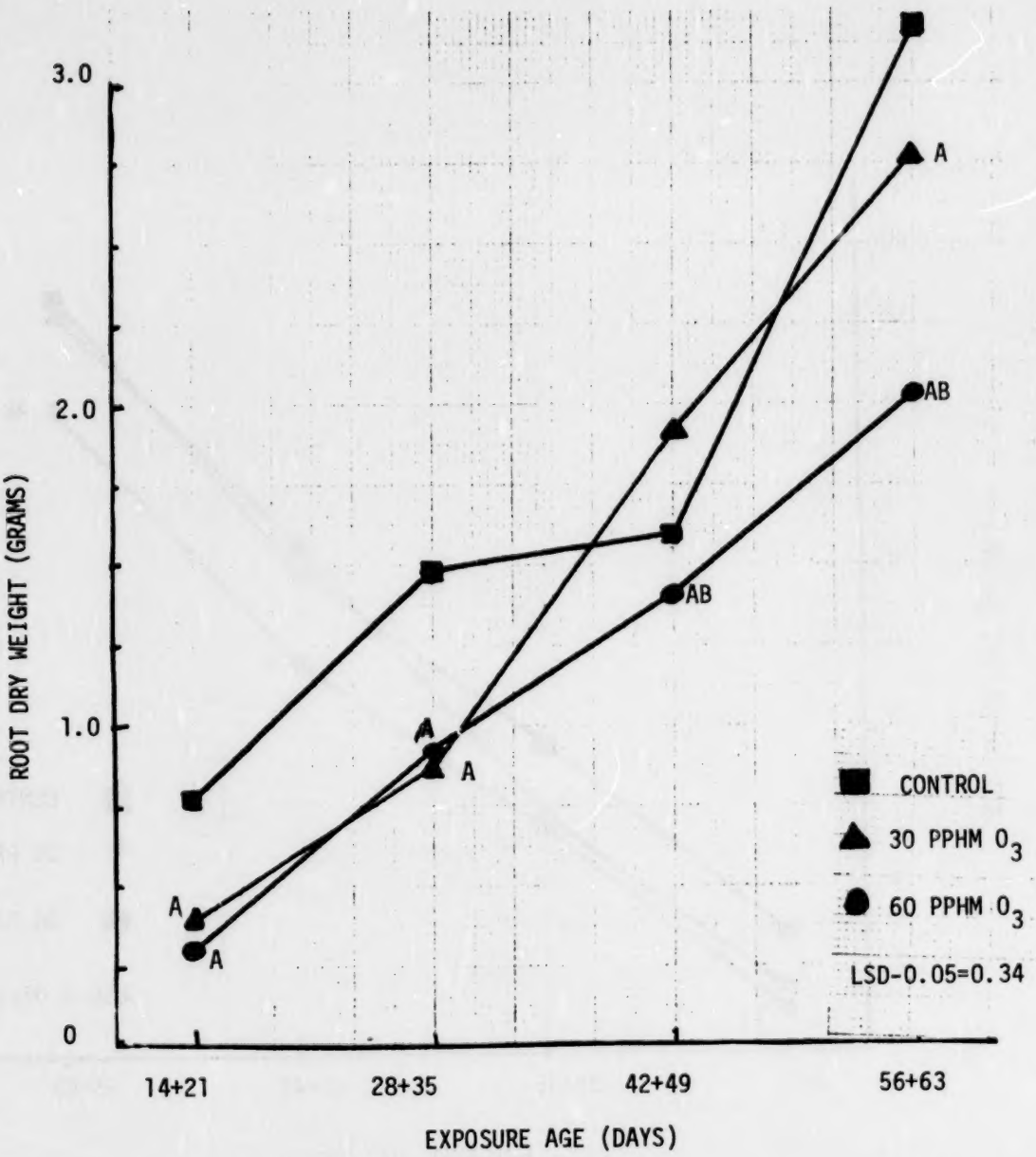

Figure 2. The effect of two 2-hr ozone exposures on the root dry weight of ladino clover.

A.-significant difference between control and test.

B.-significant difference between 30 and 60 pphm ozone.

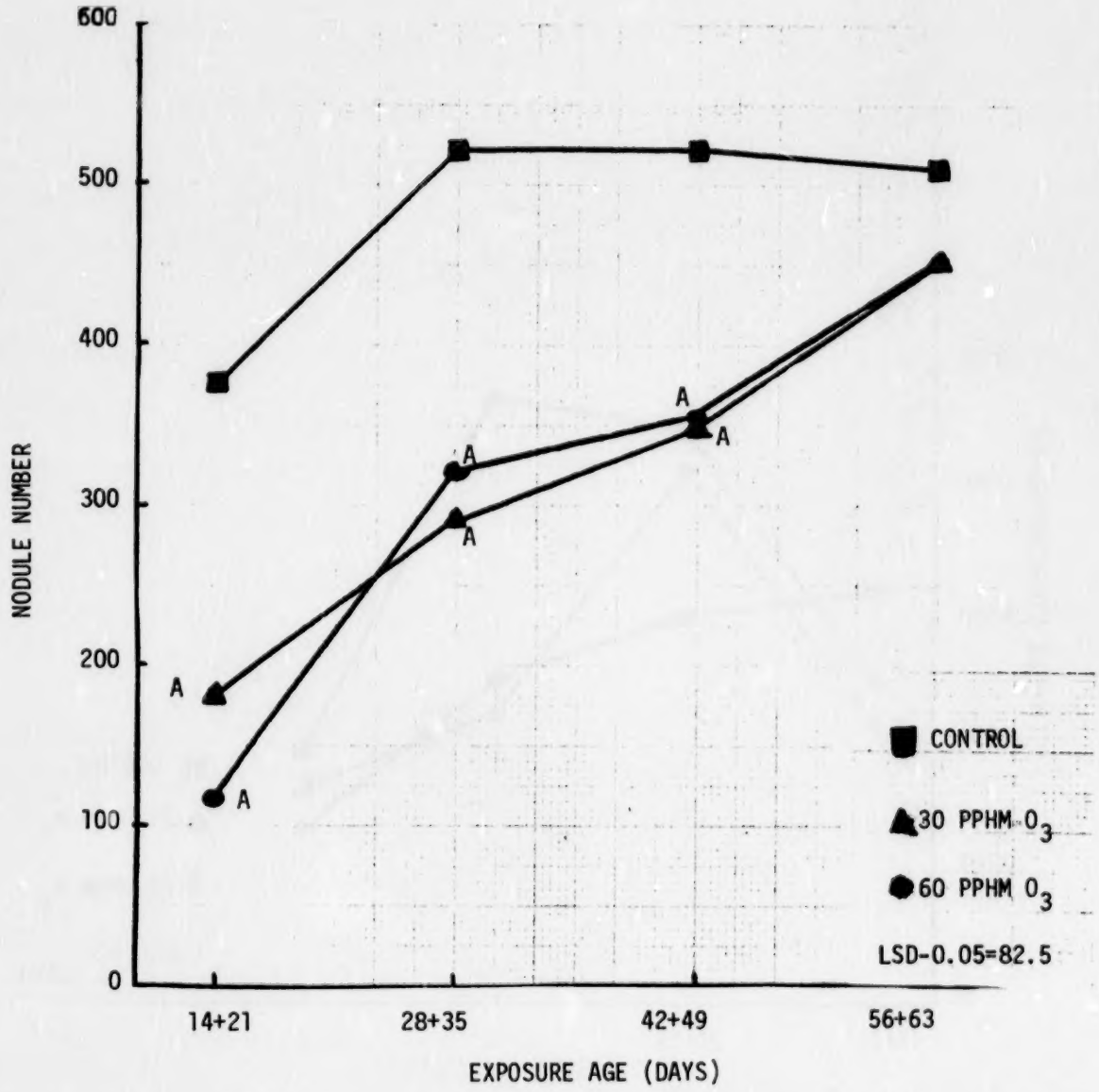

Figure 3. Changes in nodulation of ladino clover plants following two 2-hr. ozone exposures.

A.-significant difference between control and test.

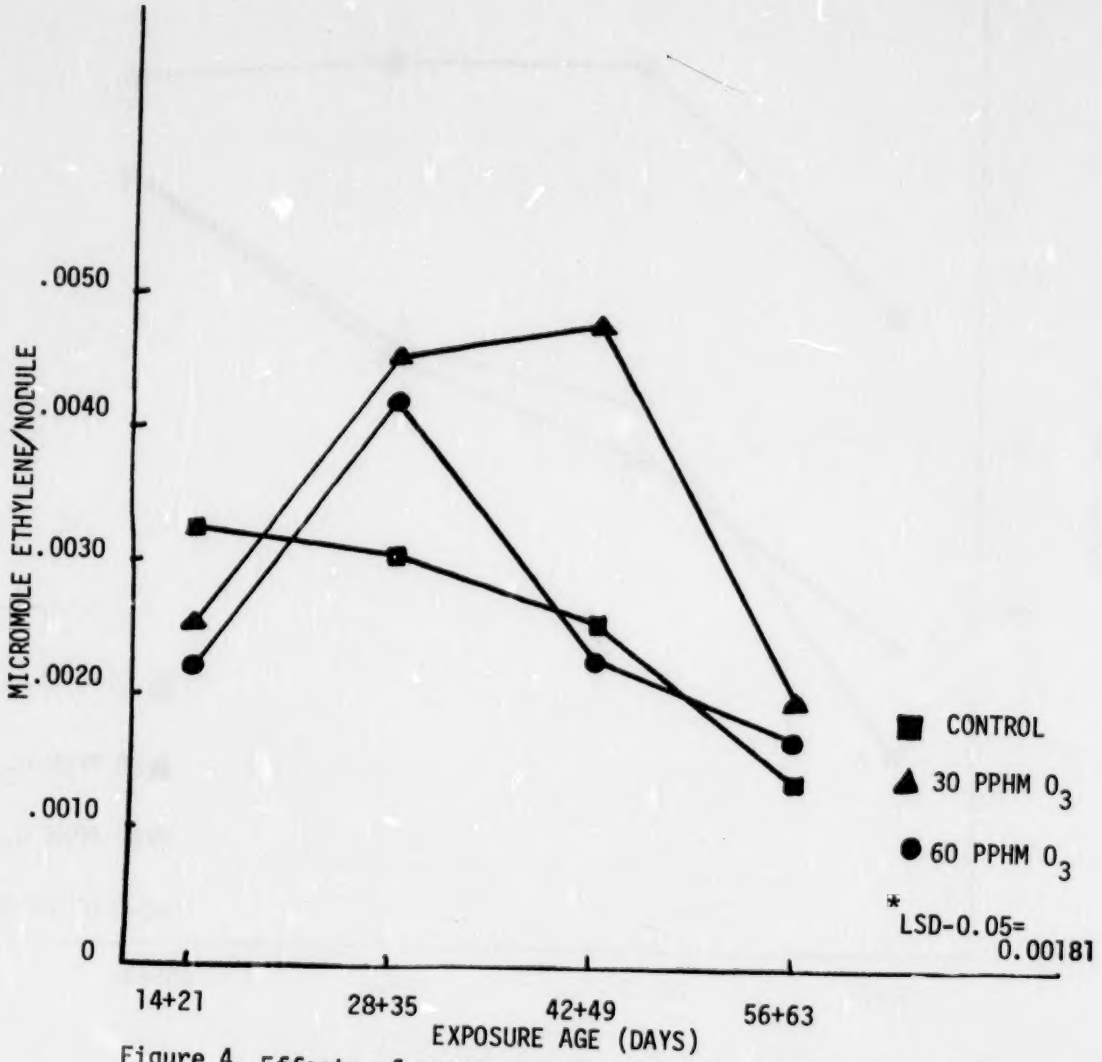

Figure 4. Effects of ozone exposures on nitrogenase activity/nodule of ladino clover.

*LSD for analysis of first 2 exposure ages only.

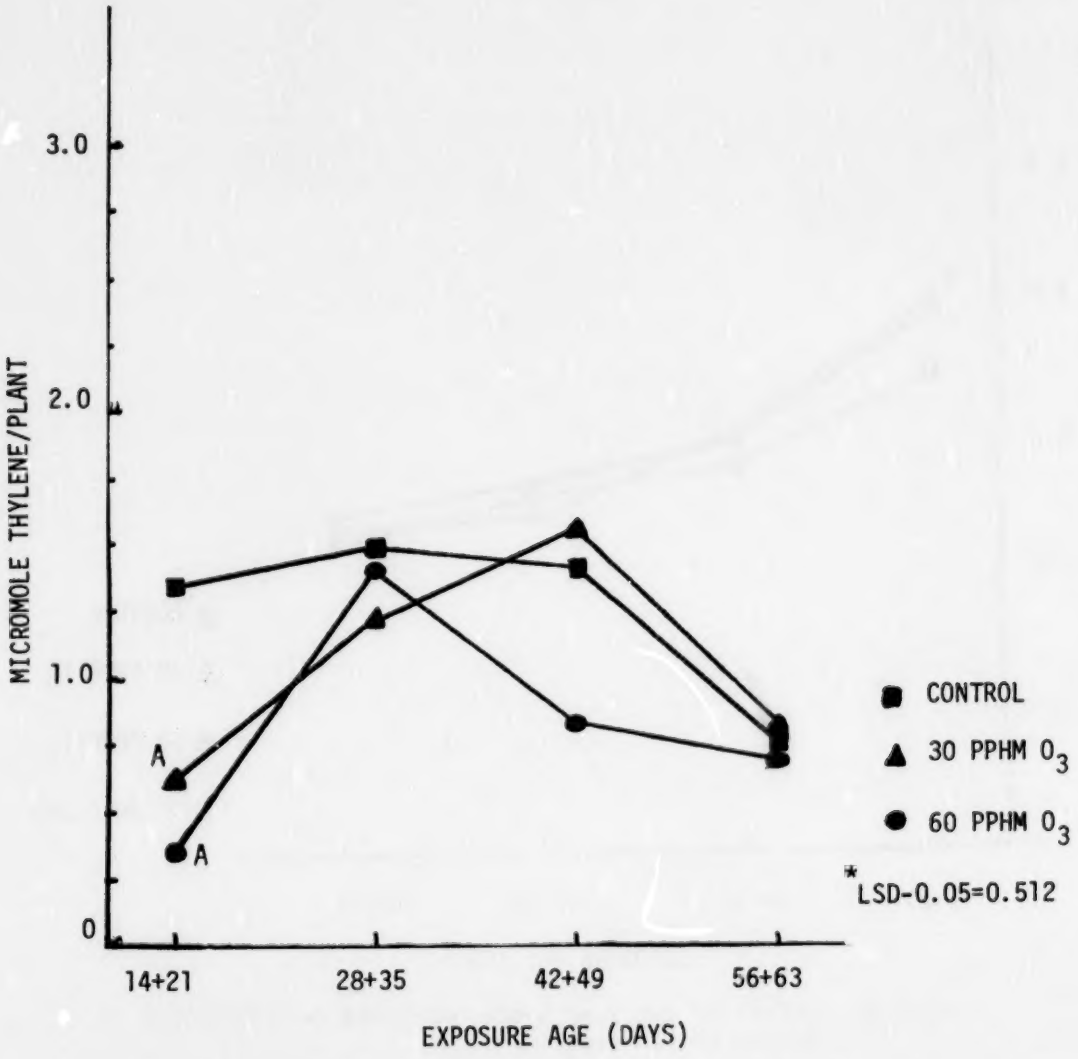

Figure 5. Changes in nitrogenase activity/plant of ladino clover following ozone exposure.

A.-significant difference between control and test.

*LSD for analysis of first 2 exposure ages only.

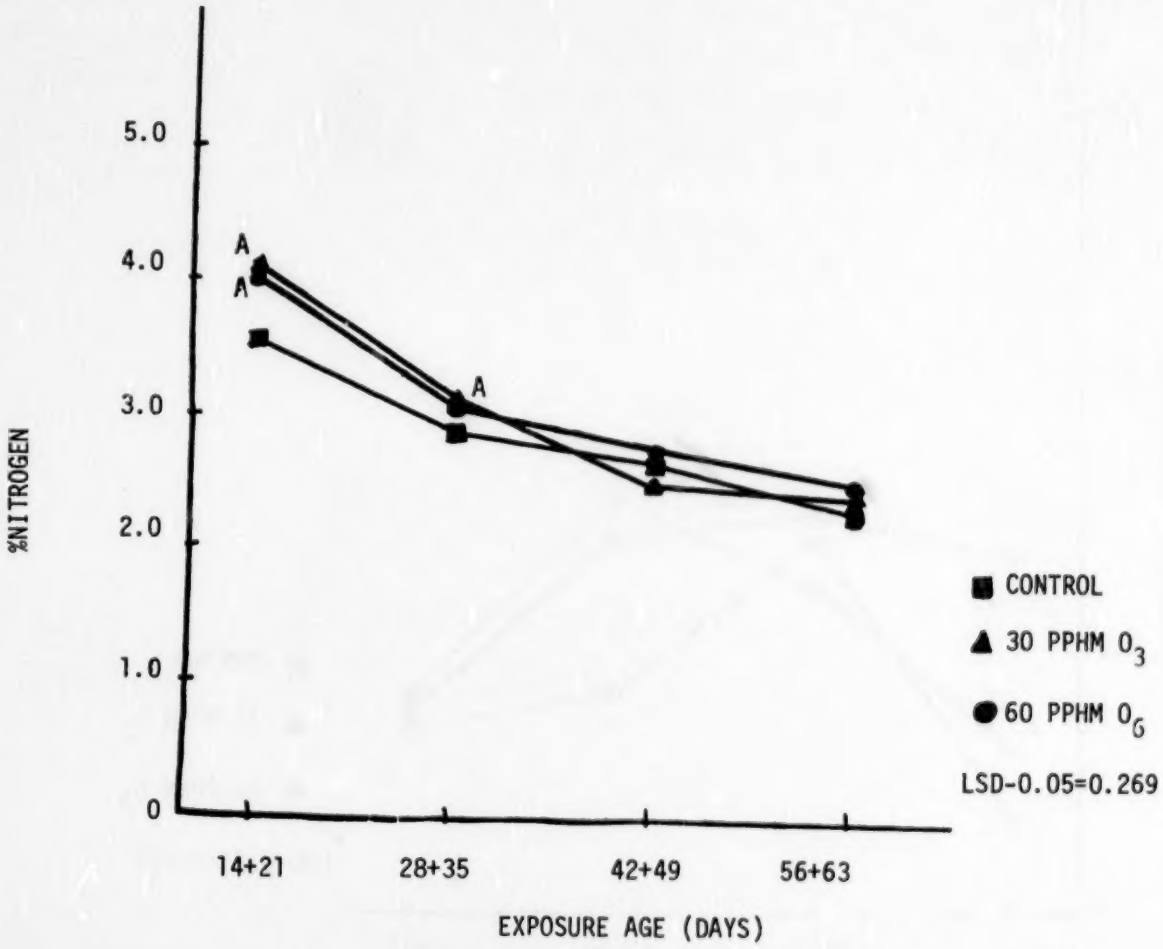

Figure 6. Effect of two 2-hr ozone exposures on % Nitrogen of ladino clover tops.

A.-significant difference between control and test.

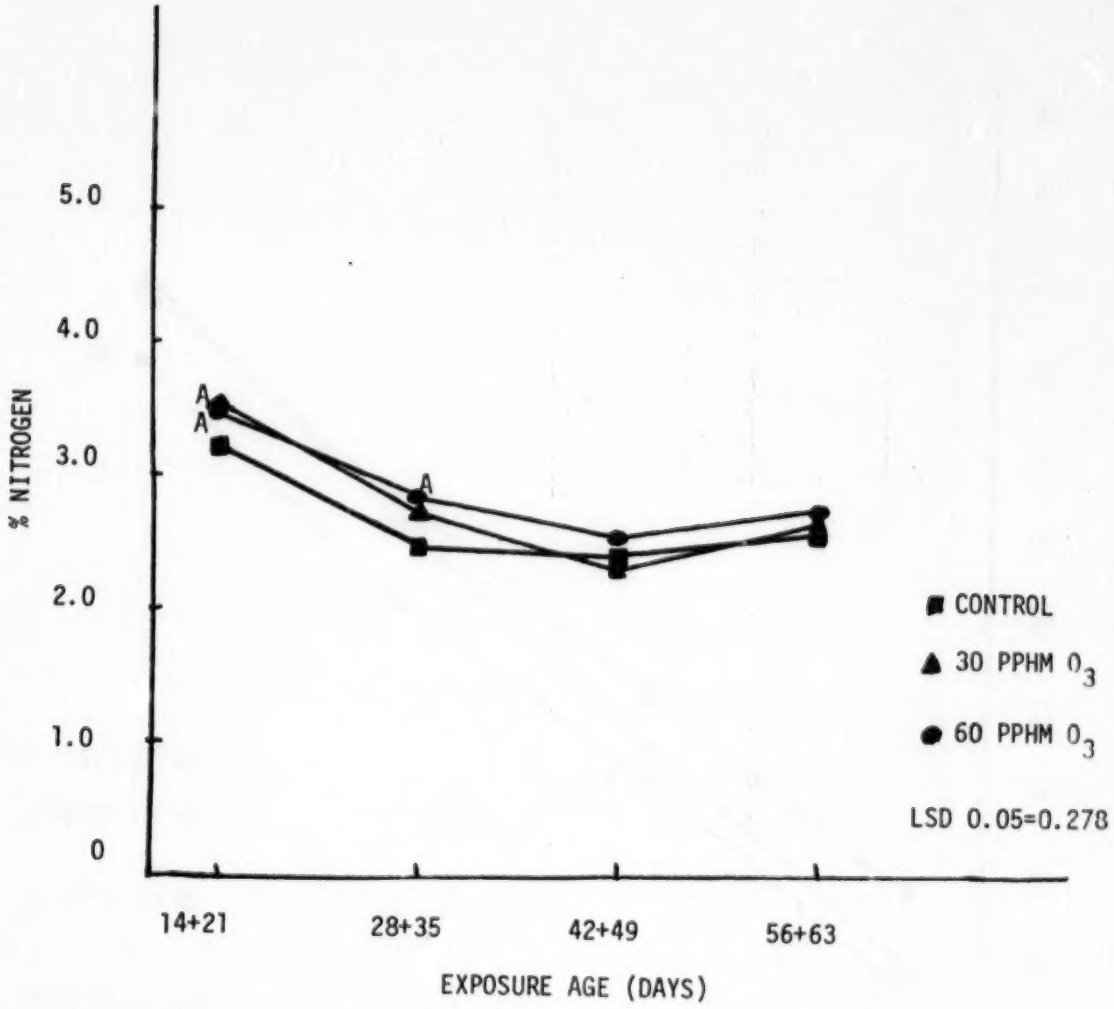

Figure 7. Effect of two 2-hr ozone exposures on % Nitrogen of ladino clover roots.

A.-significant difference between control and test.

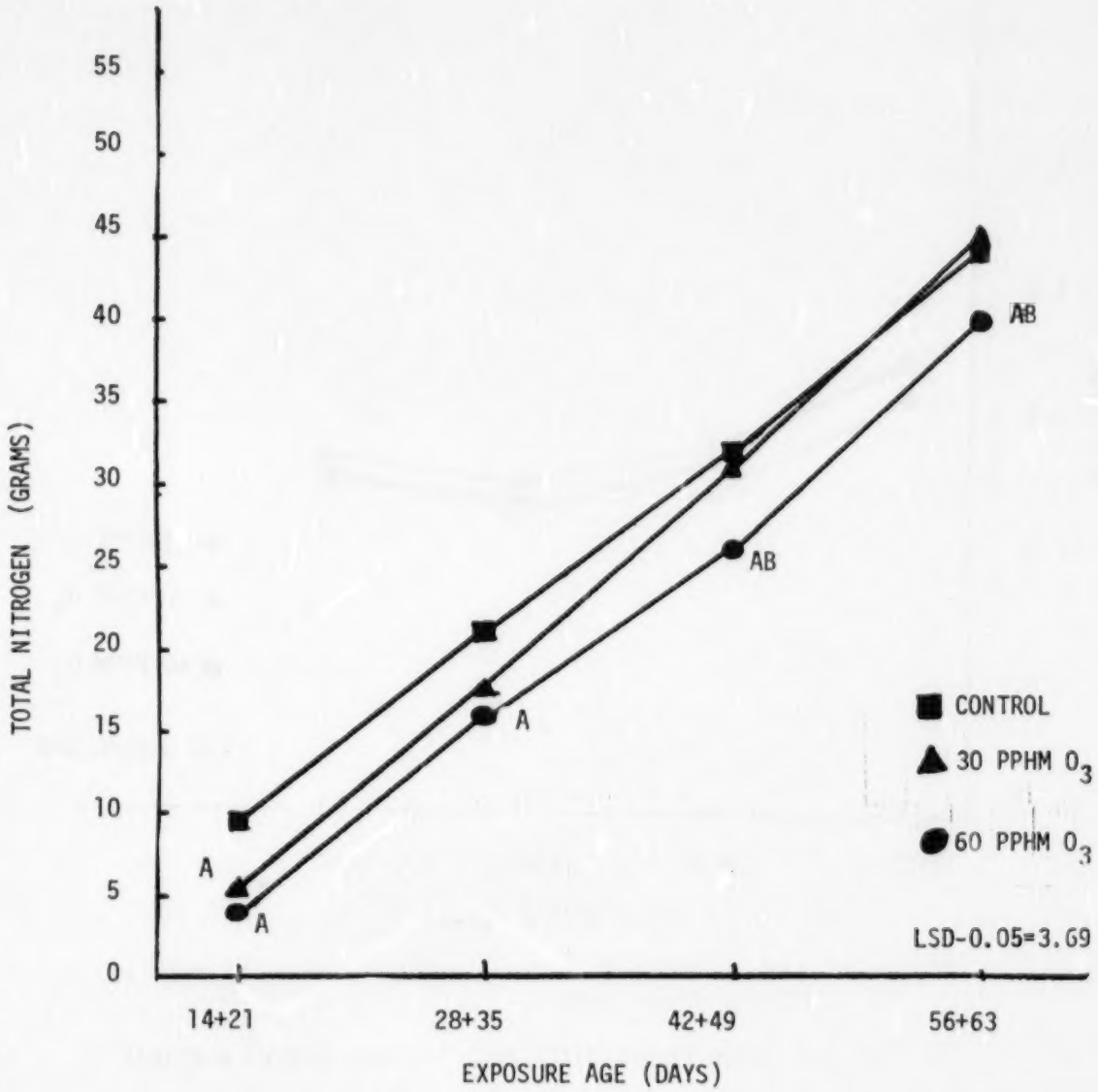

Figure 8. The effect of two 2-hr ozone exposures on the total N content of ladino clover tops.

A.-significant difference between control and test.

B.-significant difference between 30 and 60 pphm ozone.

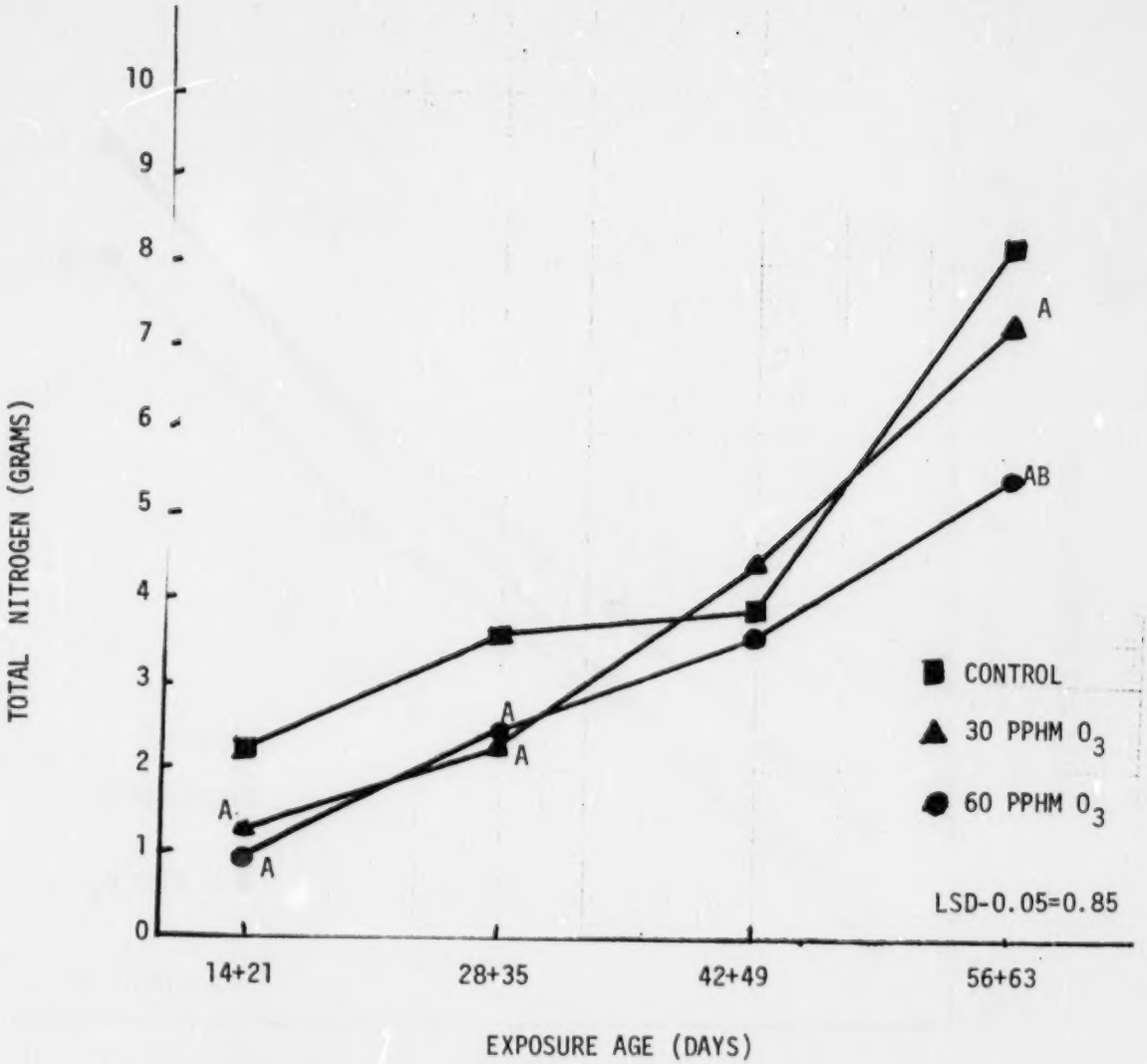

Figure 9. The effect of two 2-hr ozone exposures on the total N content of ladino clover roots.

A.-significant difference between control and test.

B.-significant difference between 30 and 60 pphm ozone.

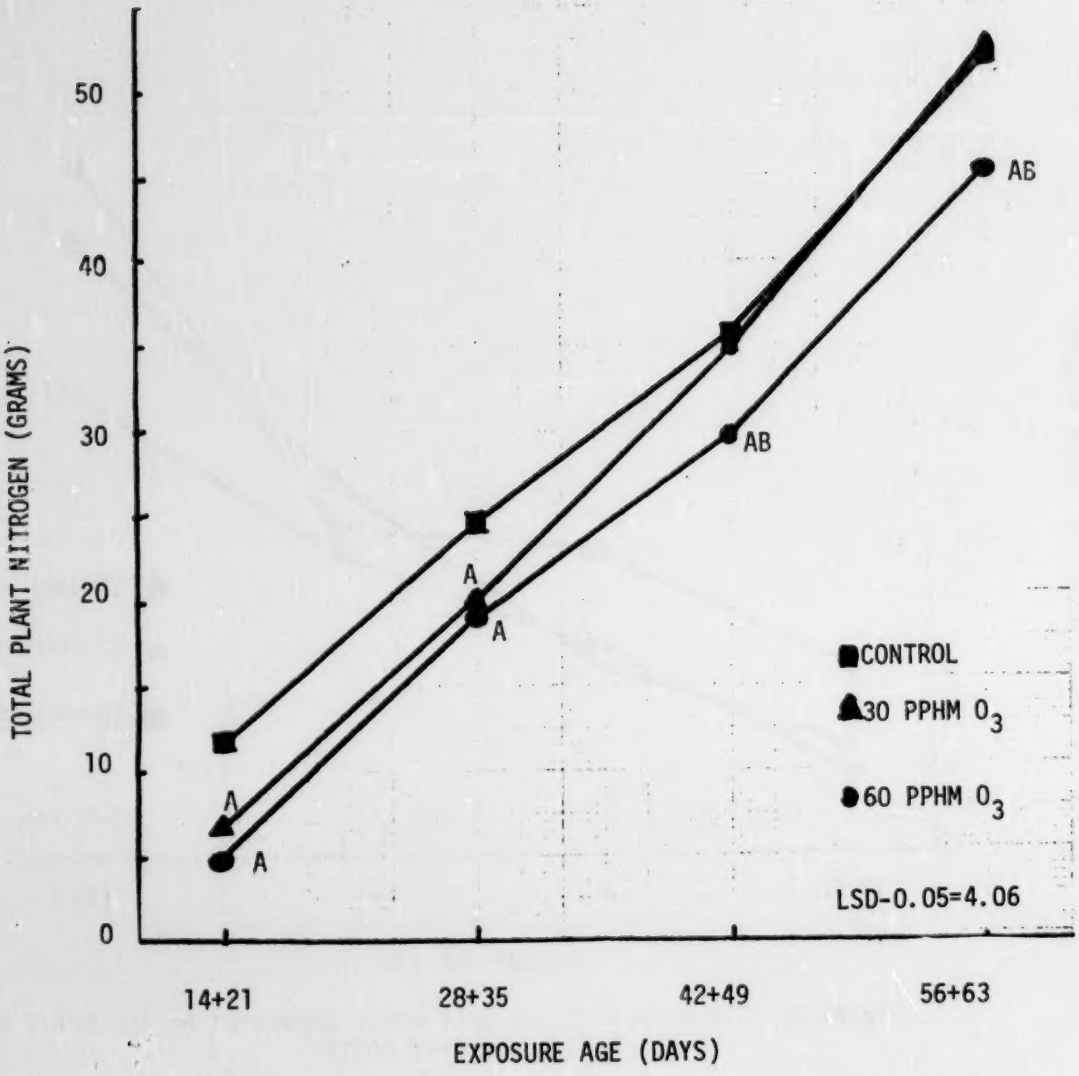

Figure 10. The effect of two 2-hr ozone exposures on the total plant nitrogen of ladino clover.

A.-significant difference between control and test.

B.-significant difference between 30 and 60 pphm ozone.

Table 1 [Ex 2] Caloric Content, Top Dry Weight, Root Dry Weight and Nodule Number of Ladino Clover Exposed to Ozone or Carbon Filtered Air.

	TDW (gm)	RDW (gm)	NODULE (#)	TPCAL (cal/gm)	RTCAL (cal/gm)
Harvest 1					
Non-Exposed	1.01 c**	0.35 b	137 bc	3980 bc	3621 a
30 pphm O_3	0.62 d	0.21 cd	105 c	3951 c	3655 a
60 pphm O_3	0.59 d	0.19 d	96 c	3919 c	3683 a
Harvest 2					
Non-Exposed	2.66 a	0.61 a	239 a	4136 a	3838 a
30 pphm O_3	1.36 b	0.28 bc	169 b	4031 b	3734 a
60 pphm O_3	0.93 c	0.19 d	99 c	3844 d	3712 a
LSD 0.05	0.28	0.07	58	70	154 (NS)*

* non significant

**means followed by the same letter are not statistically significant at the 5% level of probability. [N-10].

Table 2a [Ex 3] Phenol Content, Nitrogen Content, Top Dry Weight, Root Dry Weight and Nodule Number of Ladino Clover Exposed to Ozone and Carbon-Filtered Air.

	TDW (gm.)	RDW (gm.)	NODULE (#)	NIT* TOPS (mg)	NIT ROOTS (mg)	NIT TOT (mg)	PH* TOPS (mg)	PH ROOTS (mg)	PH TOT. (mg)
Non-Exposed Plants	0.78 a**	0.30a	243a	34a	11a	45a	168a	14a	182a
30 pphm 0_3	0.38 b	0.14b	137b	15b	5b	20b	85b	4b	89b
LSD 0.05	0.16	0.05	65.8	8.4	2	10	34	2	35

*NIT=Nitrogen

*PH=Phenol

**Means followed by the same letter are not statistically significant at the 5% level of probability [N=10].

Table 2b [Ex. 3] Top Dry Weight, Root Dry Weight, Nodule Number, Nitrogen Content and Phenol Content of Ladino Clover treated with Root Exudates.

	TDW (gm)	RDW (gm)	NODULE (#)	NITROGEN** TOTAL (mg.)	PHENOL** TOTAL (mg.)
Non-Exposed Plant Exudate	0.055*	0.024	51.3	0.034	0.16
30 pphm 0_3 Exposed Plant Exudate	0.0608	0.029	54.6	0.033	0.19

*no significant difference found. [N=10]

**Data not analyzed.

Table 3 [Ex 4] Top Dry Weight, Root Dry Weight, and Nodule Number of Ladino Clover exposed to ozone or carbon-filtered Air Grown Separately or in Combination (Design is such that plants interact only by way of root exudates).

	TDW (gm)	RDW (gm)	NODULE (#)
N Ex **	0.426 a*	0.144 a	53.5 a
N Ex + 30	0.443 a	0.133 a	58.8 a
N Ex + 60	0.466 a	0.135 a	49.6 ab
60	0.214 b	0.077 b	26.1 c
30	0.124 c	0.032 c	21.9 c
30 + N Ex	0.153 bc	0.047 c	33.3 bc
60 + N Ex	0.109 c	0.033 c	23.3 c
LSD 0.05	0.087	0.028	18.9

*means followed by the same letter are not statistically significant at the 5% level of probability [N=10].

** N Ex - Non exposed plant grown alone.

N Ex + 30 - Non exposed plant grown with plants exposed to 30 pphm of ozone.

N Ex + 60 - same as N Ex + 30 but plants exposed to 60 pphm instead of 30 pphm.

30 N 60 - exposed plants grown alone.

30 + N Ex - exposed plants from N Ex + 30.

60 + N Ex - exposed plants from N Ex + 60.

Table 4 [Ex 4] Root Exudates vs Rhizobium

	ZONES OF INHIBITION (mm)
N Ex Plant Exudates **	0.096 *
N Ex + 30 Exudates	0.081
N Ex + 60 Plant Exudates	0.080
30 Plant Exudates	0.065
60 Plant Exudates	0.040
Acetone	0.15
LSD 0.05	0.10 [NS]
Prob > F	0.099 c.v. = 77%

* Non significant [N=16]

** N Ex - non exposed

30 - plants exposed to 30 pphm ozone

60 - plants exposed to 60 pphm ozone

SECTION V

REFERENCES

1. Reinert, R.A., D.T. Tingey, and C.E. Koons. The Early Growth of Soybeans as Influenced by Ozone Stress. Agron. Abst. 63:148. 1971.

2. Tingey, D.T. and U. Blum. Effects of Ozone on Soybean Nodules. J. Environ. Qual 2: 341-342. 1973.

3. Kochhar, M. Phytotoxic and Competitive Effects of Tall Fescue on Ladino Clover as Modified by Ozone and/or Rhizoctomia Solani. Dissertation. 1974.

4. Andersen, R.A. and J.R. Todd. Estimation of Total Tobacco Plant Phenols by Their Bonding to Polyvinyl Phyrrolitone. Tobacco Science 12: 107-111. 1968.